

Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke

Mccabe, Dominick J.h.; Murphy, Stephen J.x.; Starke, Richard; Harrison, Paul; Brown, Martin M.; Sidhu, Paul S.; Mackie, Ian J.; Scully, Marie; Machin, Samuel J.

DOI:

[10.1016/j.jns.2014.10.035](https://doi.org/10.1016/j.jns.2014.10.035)

License:

Other (please specify with Rights Statement)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Mccabe, DJH, Murphy, SJX, Starke, R, Harrison, P, Brown, MM, Sidhu, PS, Mackie, IJ, Scully, M & Machin, SJ 2015, 'Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke', *Journal of the Neurological Sciences*, vol. 348, no. 1-2, pp. 35-40. <https://doi.org/10.1016/j.jns.2014.10.035>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

NOTICE: this is the author's version of a work that was accepted for publication in Journal of the Neurological Sciences. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Journal of the Neurological Sciences, Vol 348, Issue 1-2, January 2015, DOI: 10.1016/j.jns.2014.10.035.

Eligibility for the repository checked March 2015

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Accepted Manuscript

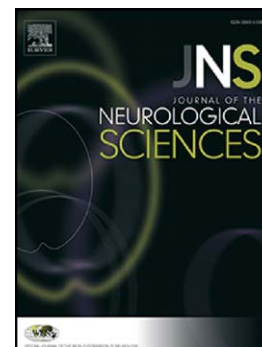
Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke

Dominick J.H. McCabe, Stephen J.X. Murphy, Richard Starke, Paul Harrison, Martin M. Brown, Paul S. Sidhu, Ian J. Mackie, Marie Scully, Samuel J. Machin

PII: S0022-510X(14)00703-5
DOI: doi: [10.1016/j.jns.2014.10.035](https://doi.org/10.1016/j.jns.2014.10.035)
Reference: JNS 13505

To appear in: *Journal of the Neurological Sciences*

Received date: 9 July 2014
Revised date: 3 October 2014
Accepted date: 27 October 2014



Please cite this article as: McCabe Dominick J.H., Murphy Stephen J.X., Starke Richard, Harrison Paul, Brown Martin M., Sidhu Paul S., Mackie Ian J., Scully Marie, Machin Samuel J., Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke, *Journal of the Neurological Sciences* (2014), doi: [10.1016/j.jns.2014.10.035](https://doi.org/10.1016/j.jns.2014.10.035)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke

Dominick J. H. McCabe PhD, FRCPI ^{*∞¶†‡}, Stephen J.X. Murphy MRCPI, MRCP UK ^{*∞},

Richard Starke PhD [†], Paul Harrison FRCPATH PhD [#], Martin M. Brown FRCP [¶],

Paul S. Sidhu MRCP FRCR [¶], Ian J. Mackie PhD, FRCPATH [†], Marie Scully FRCPATH [≈],

Samuel J. Machin FRCP ^{†≈}

Department of Neurology ^{*} and Stroke Service [∞], The Adelaide and Meath Hospital, Dublin, incorporating the National Children's Hospital, Trinity College Dublin, Ireland; Stroke Research Group [¶], and Department of Clinical Neurosciences, Royal Free Campus [‡], UCL Institute of Neurology, The National Hospital for Neurology & Neurosurgery, UK; School of Immunity and Infection [#], University of Birmingham Medical School, UK; Haemostasis Research Unit [†], Dept. of Haematology [≈], University College London, UK.

Address for Correspondence: Dr Dominick J H McCabe, Department of Neurology, Adelaide and Meath Hospital, Dublin, incorporating the National Children's Hospital, Tallaght, Dublin 24, Ireland.

Telephone: -353-1-4144217

Fax: -353-1-4143031

e-mail: dominick.mccabe@amnch.ie

Word Count (Text including acknowledgements): 3802 words

Keywords: ADAMTS13; VWF antigen; Stroke; TIA; Platelets; PFA-100[®]

Abstract

Background: Reduced ADAMTS13 activity is seen in thrombotic thrombocytopenic purpura (TTP), and may lead to accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers *in vivo*. ADAMTS13 activity and its relationship with VWF antigen (VWF:Ag) levels and platelet function in 'non-TTP related' TIA or ischaemic stroke has not been comprehensively studied.

Methods: In this prospective pilot observational analytical case-control study, ADAMTS13 activity and VWF:Ag levels were quantified in platelet poor plasma in 53 patients in the early phase (≤ 4 weeks) and 34 of these patients in the late phase (≥ 3 months) after TIA or ischaemic stroke on aspirin. Data were compared with those from 22 controls not on aspirin. The impact of ADAMTS13 on platelet function in whole blood was quantified by measuring Collagen-ADP (C-ADP) and Collagen-Epinephrine closure times on a platelet function analyser (PFA-100®).

Results: Median ADAMTS13 activity was significantly reduced in the early phase (71.96% vs. 95.5%, $P < 0.01$) but not in the late phase after TIA or stroke compared with controls (86.3% vs. 95.5%, $P = 0.19$). There was a significant inverse relationship between ADAMTS13 activity and VWF:Ag levels in the early phase ($r = -0.31$; $P = 0.024$), but not in the late phase after TIA or stroke ($P = 0.74$). There was a positive correlation between ADAMTS13 activity and C-ADP closure times in early phase patients only, likely mediated via VWF:Ag levels.

Discussion: ADAMTS13 activity is reduced and VWF:Ag expression is increased within 4 weeks of TIA or ischaemic stroke onset, and can promote enhanced platelet adhesion and aggregation in response to stimulation with collagen and ADP via VWF-mediated pathways. These data improve our understanding of the dynamic haemostatic and thrombotic profiles of ischaemic cerebrovascular disease (CVD) patients, and are important in view of the potential future role that ADAMTS13 may have to play as an anti-thrombotic agent in CVD.

Introduction

Von Willebrand factor (VWF) is a multimeric plasma glycoprotein synthesised in vascular endothelial cells and megakaryocytes.(1, 2) VWF is stored as a mixture of multimers in the α -granules of platelets, and as ultra-large multimers in Weibel-Palade bodies of endothelial cells.(3) After release in response to injury or inflammation, VWF may bind to GP1b-IX-V or α IIb β 3 receptors on platelets, and promote platelet adhesion, aggregation and subsequent thrombus formation. If not consumed immediately, ultra-large VWF is cleaved by ADAMTS13-like And Metalloprotease with Thrombospondin type I repeats 13 (ADAMTS13) into smaller, less adhesive multimers that circulate in plasma.(2) Deficiency of ADAMTS13 activity is observed in thrombotic thrombocytopenic purpura (TTP),(4) and is associated with accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers. Murine stroke models have suggested that reduced ADAMTS13 activity significantly aggravates ischaemic brain injury.(5, 6) A number of studies have identified either low ADAMTS13 antigen levels or activity in coronary artery disease (7-9) or at a single timepoint after ischaemic stroke,(10-12) but other studies have not confirmed these findings.(1) Increased VWF levels have been reported in ischaemic cerebrovascular disease (CVD) patients,(1, 13-15) and may cause platelet hyper-reactivity and shortened 'closure times' on a high shear stress-dependent platelet function analyser called the PFA-100[®].(16-18) ADAMTS13 activity has been shown to be inversely correlated with VWF antigen levels,(19) but to our knowledge, the impact of ADAMTS13 activity on platelet function on the PFA-100[®] in patients in both the early and late phases after TIA and stroke has not been studied.

The aims of this study were to assess ADAMTS13 activity in both the early and late phases after ‘non-TTP related’ TIA or ischaemic stroke, to assess the relationship between ADAMTS13 activity and VWF antigen (VWF:Ag) levels in CVD patients, and to determine whether ADAMTS13 activity independently influences platelet reactivity in CVD. We hypothesised that ADAMTS13 activity would be reduced in CVD patients versus controls, and that ADAMTS13 activity would correlate with VWF:Ag levels and platelet reactivity in ischaemic CVD.

Methods

CVD Patient Inclusion Criteria

Consecutive eligible patients referred by General Practitioners or Consultant colleagues to our secondary and tertiary referral stroke prevention outpatient clinic or inpatient stroke neurology or liaison neurology service at the National Hospital for Neurology and Neurosurgery, University College London Hospitals, UK were recruited to this prospective pilot observational analytical case-control study. Patients were included if they were older than 18 years of age, had experienced a TIA or ischaemic stroke within the preceding 4 weeks (early phase), had been commenced on aspirin, and were likely to be available for clinical and laboratory follow-up at least 3 months after symptom onset (late phase). The study was approved by the Local Research Ethics Committees of the participating Hospitals. Written informed consent (or written assent, where appropriate) was obtained in all cases.

CVD Patient Exclusion Criteria

We excluded patients who were on another antiplatelet or non-steroidal anti-inflammatory drug in combination with aspirin, or those receiving heparin or warfarin. Patients were also excluded if they had a history of primary intracerebral haemorrhage, myocardial infarction within the preceding 3 months, on-going unstable angina, unstable symptomatic peripheral vascular disease, major surgery or systemic haemorrhage within the preceding 3 months, or if they had systemic vasculitis, underlying neoplasia, or a known bleeding or clotting diathesis, including TTP.

One examiner (DJHM) clinically assessed all subjects at each timepoint, and information regarding vascular risk factors, smoking status, alcohol intake, and medication use was collected prospectively. Results of routine haematological, coagulation, biochemical and blood glucose testing were collected prospectively. All CVD patients had a brain CT or MRI, and colour Doppler ultrasound examination of carotid¹⁸ and vertebral arteries. Some patients underwent extra- or intra-cranial magnetic resonance angiography or intra-arterial catheter angiography, if deemed appropriate by the treating physician. A chest radiograph and an electrocardiograph (ECG) were obtained in all patients. A 24-hour ECG recording was performed if paroxysmal atrial fibrillation or flutter was suspected clinically, and transthoracic \pm transoesophageal echocardiography was also carried out if a cardioembolic cause for stroke or TIA was suspected, or when other investigations were uninformative, as previously described.(18) The underlying mechanism responsible for the TIA or ischaemic stroke was categorised according to slight modifications of the TOAST classification as large artery atherosclerotic, lacunar, cardio-

embolic, other determined, and undetermined aetiology (Table 2).(20) For the purpose of this study, large artery atherosclerotic TIA or stroke specifically referred to TIA or stroke in the vascular territory supplied by a **severe (>70%)** ipsilateral extracranial carotid stenosis or occlusion to comply with inclusion criteria for other ongoing collaborative studies in our laboratory. The late phase follow-up in the large artery atherosclerotic subgroup was performed ≥ 3 months after carotid surgery or endovascular treatment, unless this treatment had been delayed for at least 3 months after the initial event.

Control Subjects Inclusion and Exclusion Criteria

Control subjects were recruited from the staff at The Haemostasis Research Unit, University College London Hospitals, from the local population, and from amongst spouses of patients and control subjects. Subjects were excluded from the control group if they had a history of known vascular disease, or evidence of asymptomatic severe (> 70%) carotid or vertebral artery stenosis on colour Doppler ultrasound imaging. Otherwise, the exclusion criteria were identical to those applied to the patient group. It was expected that control subjects would have a different vascular risk profile to the patient population.

Blood Sampling and Laboratory Tests

All subjects were rested for at least 20 minutes before venepuncture, and free-flowing blood was collected using a 21-G Butterfly needle (Venisystems™, Abbott, Ireland) and a Vacutainer® system with a luer adaptor (Becton-Dickinson Vacutainer Systems, UK). For all studies, the tourniquet was released during collection of the first 4ml of blood into a sterile Vacutainer tube

containing 7.2mg of K_2 EDTA or 0.054ml of 15% K_3 EDTA. This sample was used for measurement of a full blood count (FBC). Four further 4.5ml samples were collected into sterile Vacutainer tubes containing 0.5ml of 3.2% buffered sodium citrate. The first 4.5ml citrate-anticoagulated whole blood sample was used for measurement of platelet reactivity and closure times on the PFA-100[®] in response to stimulation with Collagen-ADP (C-ADP) and Collagen-Epinephrine (C-EPI) between 2 and 2.5 hours after venepuncture, as previously described.(18) The maximum closure time recorded by the device is 300s, and we arbitrarily defined closure times >300s as 301s.(18)

The next two citrated samples were used to prepare double-centrifuged (2000g x 15 minutes) platelet-poor plasma (PPP) that was immediately stored at -70°C for later analysis. VWF antigen levels were measured in PPP with an automated latex agglutination assay (STA Liatest VWF, DiagnosticaStago, Asnieres, France), modified for use on a Sysmex CA-1500[™] fully-automated coagulometer (Sysmex UK Ltd, Milton Keynes, UK) using Coagulation Reference Plasma (Technoclone, Vienna, Austria) to prepare a standard curve.(18)

ADAMTS13 activity in double-centrifuged PPP was quantified using a collagen binding assay described by Yarranton *et al.*, 2005, and modified from Gerritsen *et al.*, 1999.(21, 22) Samples were analysed in batches at the end of the study period, with no sample stored for longer than 72 months. Freeze-thawing of samples was avoided by storing samples in multiple, separate aliquots. After dilution of the plasma, ADAMTS13 was activated by incubation at 37°C for 5 min with 10 mmol/L barium chloride and 0.9mmol/L Pefabloc SC[®] (Sigma-Aldrich, Poole, UK). Immediately after activation, VWF concentrate (final concentration = 0.561 IU/mL; French Laboratory of Fractionation and Biotechnology, Lille, France) was added and the samples

incubated overnight in the presence of urea (final concentration = 1.36 mol/L) at 37°C. The reaction was stopped by adding disodium-EDTA (final concentration = 0.1 mol/L). Samples were then diluted and added to a collagen-coated microtitre plate. The residual high molecular weight multimers of VWF bound to collagen on the plates and were detected with an anti-VWF antibody conjugated to HRP (Dako), followed by detection with 1,2-ortho-phenylenediamine substrate (Sigma-Aldrich). The results were expressed relative to the ADAMTS13 activity of pooled normal plasma. Although not performed under typical physiological conditions, this assay has been shown to be sensitive and reproducible, with a typical normal range of $101.6 \pm 49.4\%$, and intra-and inter-assay coefficients of variation calculated as 3% and 7.2%, respectively.(23)

In an exploratory sub-study, all patients with **reduced ADAMTS13 activity** (ADAMTS13 activity at least 2 standard deviations below values obtained from PNP in our laboratory i.e. ADAMTS13 activity < 66%) had additional commercial assays performed to quantify plasma ADAMTS13 antigen levels, and to look for the presence of auto-antibodies to ADAMTS13. Antigen levels were measured with an Imubind® ADAMTS13 ELISA kit, and anti-ADAMTS13 IgG auto-antibodies were quantified with an Imubind® ADAMTS13 auto-antibody ELISA kit (American Diagnostica, Stamford, CT, USA), according to the manufacturer's instructions.(24)

Statistical Methodology

The Mann–Whitney U-test was used for comparison of median values, an unpaired t-test for comparison of mean values, and one-way ANOVA for comparison of ADAMTS13 activity

between stroke subtypes, where appropriate. Analysis of covariance (ANCOVA) was employed to examine the relationship between ADAMTS13 and VWF:Ag levels, and to control for the effects of potential confounding variables, where appropriate. Linear regression analysis examined the relationship between plasma VWF:Ag levels and PFA-100[®] closure times. Stepwise regression assessed the impact of ADAMTS13 activity on PFA-100[®] closure times whilst controlling for VWF:Ag levels. $P < 0.05$ was considered statistically significant. All statistical calculations were performed with Minitab 6.0 for Windows.

Results

Between May 1999 and July 2001, 53 patients were assessed in the early phase (range: 0 – 27 days) after TIA ($N = 3$) or ischaemic stroke ($N = 50$) with complete clinical and laboratory follow-up data available in 34 of these patients in the late phase (≥ 3 months) after symptom onset (range: 90-725 days). The interval between symptom onset and venepuncture was lower than in other studies of ADAMTS13 in ischaemic stroke, (1, 10-12) with mean times to venepuncture of 7.85 (± 5.3) days in early phase CVD patients, and 160.3 (± 76.8) days in the late phase CVD group. The clinical details of the study subjects are outlined in tables 1 and 2.

CVD patients were older ($P < 0.001$), and as expected, vascular risk factors were more common in CVD patients than controls (table 1). In particular, hypertension and a definite diagnosis of hyperlipidaemia were more common in early and late phase CVD patients, and the proportion of smokers was higher in late phase patients than controls. No study subject had a platelet count $< 150 \times 10^9/L$, and none had any other clinical features of TTP.

Table 1: Demographic Data and Vascular Risk Factor Profile of Study Subjects at Enrolment

Characteristic	Early (n = 53)	P Value	Late (n=34)	P Value	Controls (n=22)
Mean age (years)	66.7 ±[12.71]	0.001	66.29 ±[11.04]	0.0052	58.14 ± [10.64]
Sex (% Female)	47%	0.059	44%	0.06	68%
Stroke at presentation	50 (94.3%)	0.966	49 (92.45%)	N/A	0
Prior Stroke/TIA	14(26.4%)	<0.001	3(8.8%)	0.271	0
Ischaemic Heart Disease	8(15.1%)	0.869	4(11.8%)	0.838	3(13.6%)
Hypertension	34(64.2%)	<0.001	20(58.8%)	<0.001	2(9.1%)
Diabetes Mellitus	11(20.8%)	0.027	1(2.9%)	0.310	0
Atrial Fibrillation /Flutter	5(9.4%)	0.313	2(5.9%)	0.514	2(5.9%)
Prior DVT/PE	3(5.7%)	0.627	3(8.2%)	0.973	2(9.1%)
Peripheral Vascular Disease	11(20.8%)	0.027	10(29.4%)	0.004	0
Hyperlipidaemia*	21(54.7%)	<0.001	20(58.8%)	<0.001	2(9.1%)
Migraine	6(11.3%)	0.171	3(8.8%)	0.271	0
MRS [†]	3	0.048	2	N/A	N/A
Smoking at Enrolment	15(28.3%)	0.807	10(29.4%)	0.849	7 (31.8%)
Ex-smoker	19(35.85%)	0.128	14(41.2%)	0.248	5 (22.7%)
Never smoker	19(35.85%)	0.807	10(29.4%)	0.262	10 (45.45%)
Statin Therapy	21(39.6%)	<0.001	17(50%)	<0.001	0
Family History Stroke	19(35.9%)	0.620	15(44.1%)	0.922	10(45.5%)
Stroke on Aspirin	20(37.7%)	N/A	10(29.4%)	N/A	N/A
Median Daily Aspirin Dose (Range)	150mg (75 – 300)	N/A	75mg (75 – 300)	N/A	N/A

Legend for Table 1: Values are means [\pm SD] or absolute values(percentages in parentheses where appropriate), unless otherwise stated; *Hyperlipidaemia = total cholesterol >5.0 mmol/L or LDL >3.5 mmol/L. P values refer to differences between early or late phase patients and controls. Significant P values are highlighted in bold. [†]Median Modified Rankin Scale scores;

Table 2: TOAST subtyping in early and late phase CVD patients

Stroke Subtype	Early (n = 53)	Late (n = 34)
Large Artery Atherosclerotic	11 (20.75%)	6 (17.6%)
Cardioembolic	11 (20.75%)	9 (26.5%)
Lacunar	16 (30.2%)	12 (35.3%)
Other determined or undetermined aetiology	15 (28.3%)	7 (20.6%)

Table 2:TOAST subtyping in early and late phase CVD patients. Values are absolute numbers with percentages in parentheses.

Median ADAMTS13 activity was significantly reduced in the early phase after TIA or stroke compared with controls (71.96% vs. 95.5%, $P < 0.01$; figure 1). After controlling for differences in hypertension, hyperlipidemia, smoking status, and statin use between groups, **significant differences in ADAMTS13 activity between early phase CVD patients and controls persisted** ($P < 0.02$). There was no significant difference in ADAMTS13 activity between late phase CVD patients and controls (86.03% vs. 95.5%, $P = 0.19$; figure 1), even after controlling

for the above factors ($P = 0.561$). *Post-hoc* analysis indicated that there was no significant difference in ADAMTS13 activity between TIA ($N = 3$) and ischaemic stroke patients ($N = 50$; $P = 0.658$), but the number of subjects in the TIA subgroup was far too small to make any definitive conclusions, and this study was not designed to address such a subgroup analysis.

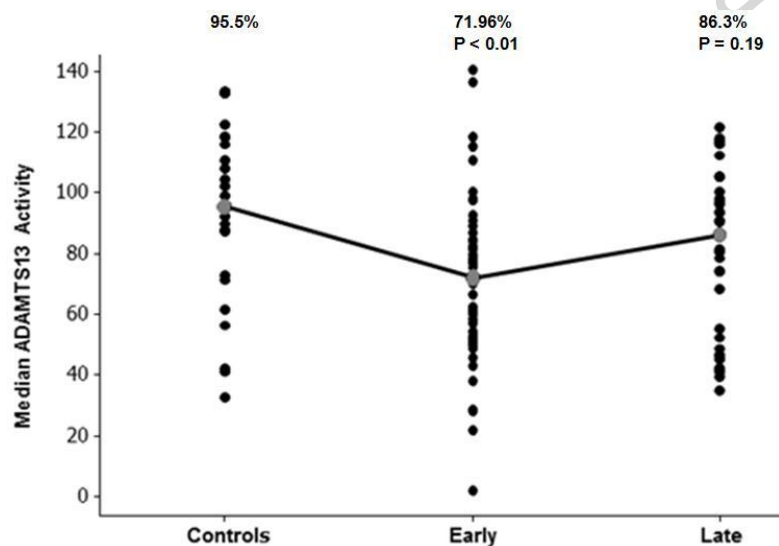


Figure 1: Comparison of median ADAMTS13 levels between controls and early and late phase CVD patients. P values refer to comparisons between patients and controls.

There was no significant correlation between ADAMTS13 activity and age in our patient population in the early phase ($r = -0.22$, $P = 0.12$), nor was there any significant difference in ADAMTS13 activity between different stroke subtypes (Tukey ANOVA, $P = 0.50$).

As reported previously,(18) VWF:Ag levels were higher in the early (201.83 IU/dl, $P < 0.001$; figure 2) and late phases after TIA or stroke (175.38 IU/dl; $P < 0.001$) than in controls (123.87 IU/dl; figure 2).

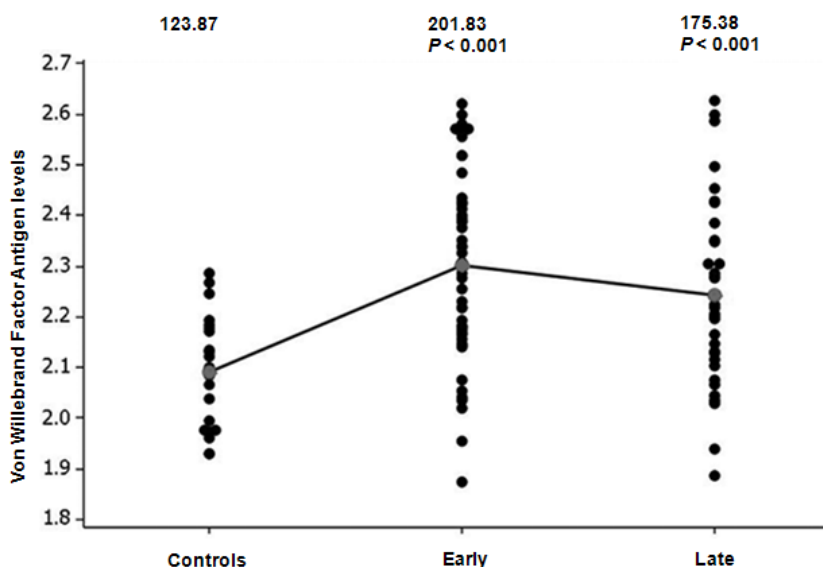


Figure 2: Comparison of mean VWF antigen levels (IU/dl) between controls and early and late phase CVD patients. P values refer to comparisons between controls and patients.

In our CVD population, there was a distinct inverse relationship between ADAMTS13 activity and VWF:Ag levels in the early phase ($r = -0.31$, $P = 0.024$; figure 3) but not in the late phase after symptom onset ($r = -0.2$, $P = 0.738$).

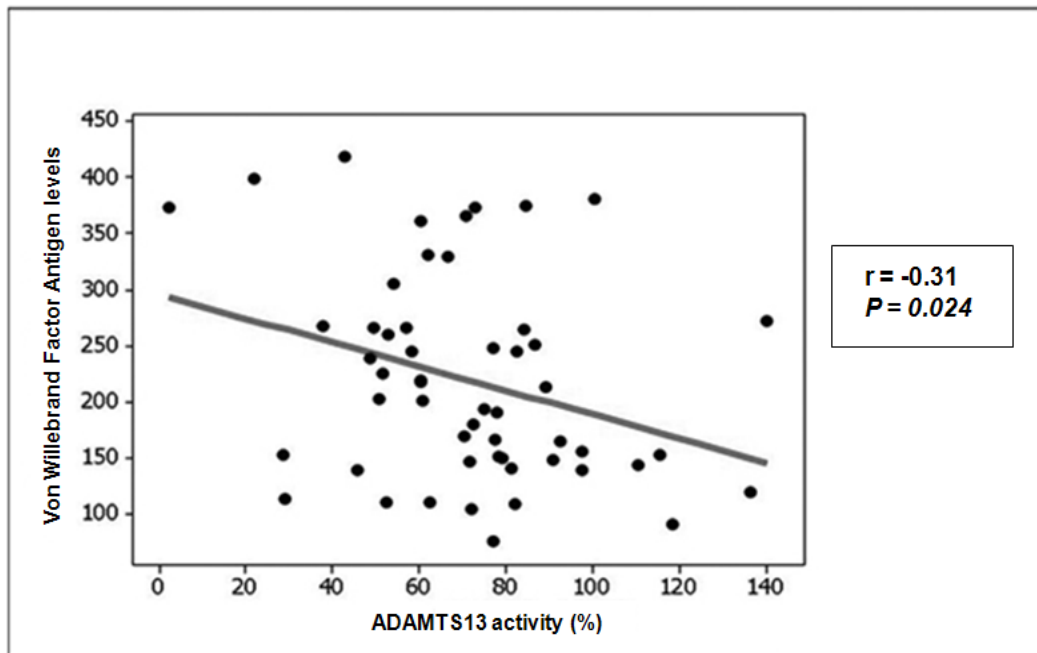


Figure 3: Relationship between VWF:Ag levels (IU/dl) and ADAMTS13 activity in the early phase after TIA or ischaemic stroke

There was a positive correlation between ADAMTS13 activity and C-ADP closure times ($r = 0.35$, $P = 0.012$) but not C-EPI closure times ($r = 0.24$; $P = 0.092$) on the PFA-100[®] in the early CVD group, suggesting that reduced ADAMTS13 activity might be associated with shorter C-ADP closure times, and hence platelet hyper-reactivity to collagen-ADP early after TIA or stroke. However, when stepwise logistic regression analysis was performed to control for the influence of VWF:Ag levels on the potential relationship between ADAMTS13 activity and C-ADP closure times, the relationship became non significant ($P = 0.066$).

Of the 53 early phase patients, 23 had reduced ADAMTS13 activity $< 66\%$ of PNP in our laboratory, but none of these patients had reduced ADAMTS13 antigen levels in the early or late

phase after symptom onset. Three of these 23 patients had elevated anti-ADAMTS13 IgG auto-antibody titres (defined as > 9.6 AU/dl) in the early phase, but only one of these patients had persistent anti-ADAMTS13 IgG auto-antibodies in the late phase.

Discussion

This novel pilot study has shown that ADAMTS13 activity is significantly reduced in the early but not in the late phase after TIA or ischaemic stroke. Our early phase samples were collected sooner after symptom onset than in prior published studies in CVD patients (mean time to blood sampling was just over 1 week; (range: 0-27 days), (1,10-12) and these novel data provide unique, important insights into the dynamic profile of ADAMTS13 and its relationship with VWF:Ag early after TIA or ischaemic stroke. One must accept that the lack of significant differences between late phase patients and controls could represent a type II error due to the smaller number of subjects studied ≥ 3 months after symptom onset. However, the lack of significantly reduced ADAMTS13 activity in late phase CVD patients suggests that this is a **dynamic protease** whose activity may decrease as an ‘acute phase response’ to TIA or ischaemic stroke, but returns to levels similar to those seen in controls during late-phase follow up.

Prior studies by our group (18) and others (1, 13, 14) have clearly demonstrated that circulating VWF:Ag levels are increased in the early and late phases after TIA and stroke, especially associated with large artery atherosclerosis. Population-based studies have also shown that ADAMTS13 activity is inversely correlated with VWF:Ag titres,(1, 19) is reduced in renal failure, in patients on dialysis and in acute inflammatory states, but is elevated in patients with

cirrhosis.(19) Our pilot hospital-based study is in agreement with these population-based data (19) because we also found that there was an inverse relationship between ADAMTS13 activity and VWF:Ag levels in early phase CVD patients. However, one cannot comment on the relationship between ADAMTS13 activity and levels of ‘individual fractions’ of circulating VWF multimers following TIA or stroke because our VWF assay measured overall VWF:Ag levels and did not differentiate between smaller, larger and ultra-large VWF multimers.

As stated above, previous studies have investigated whether low ADAMTS13 activity could be a risk factor for CVD or ischaemic heart disease. (7-12) Desmopressin infusion induces the secretion of very large VWF multimers into the plasma,(25) producing plasma ULVWF levels similar to those seen with acute tissue hypoxia *in vivo*.(15) *In vivo* testing of healthy volunteers revealed a steep decline in ADAMTS13 activity after desmopressin infusion suggesting that the protease is consumed as it cleaves very large VWF multimers.(25) These desmopressin-induced effects also occur in systemic inflammation (26) which has been noted in ischaemic stroke.(27) Our study revealed initial low ADAMTS13 activity and a distinct inverse relationship between VWF:Ag levels and ADAMTS13 activity only in the early phase after TIA or ischaemic stroke. Based on our longitudinal data, the early phase findings are consistent with the hypothesis that ADAMTS13 may be consumed or inactivated as it degrades ultra-large VWF multimers(25) associated with acute cerebral or ocular ischaemia rather than reflecting a ‘pre-existing deficiency’ of ADAMTS13 antigen in our CVD population.

Because the protease targets the largest, most thrombogenic multimers of VWF which have the greatest influence on platelet adhesion and aggregation,(28) lower ADAMTS13 activity, which would be expected to be associated with increased VWF:Ag levels, was associated with

‘apparent platelet hyper-reactivity’ and shorter C-ADP closure times. Stepwise logistic regression analysis revealed that it is highly likely that the relationship between ADAMTS13 activity and PFA-100 C-ADP closure times is mediated via VWF:Ag, and that ADAMTS13 itself does not directly influence closure times. By the late phase, ADAMTS13 activity was not lower than in controls, and the clear inverse relationship between ADAMTS13 and VWF:Ag levels seen in the early phase was no longer evident. This lack of a significant relationship between these two variables may also reflect a type II error due to the smaller number of subjects with late phase data, but could represent a shift towards normal VWF multimers in plasma over time, for which ADAMTS13 has less affinity, thus resulting in normalisation of ADAMTS13 activity during late phase follow up.

The exploratory laboratory assays that were performed in the patient subgroups with low ADAMTS13 activity indicate that the findings in our early phase CVD patients cannot not explained by a primary deficiency of ADAMTS13 antigen. Because only 3 of the 23 early phase CVD patients, and only one late phase CVD patient with low ADAMTS13 activity had positive anti-ADAMTS13 IgG auto-antibodies, one can conclude that the initial reduction in ADAMTS13 activity in non-TTP-related TIA or stroke is more likely to be due to an acute phase response, and cannot predominantly be attributed to the presence of the measured auto-antibodies to ADAMTS13.(29)

This study had some limitations. The study was designed to focus on CVD patients on aspirin monotherapy who also had simultaneous PFA-100[®] testing, so one cannot comment on the potential impact of other commonly-prescribed antiplatelet regimens, such as aspirin and dipyridamole or clopidogrel monotherapy on ADAMTS13 activity in TIA and ischaemic stroke.

This issue deserves future study. Patient data were compared with controls who were not on Aspirin; however, there is no evidence from longitudinal studies that Aspirin therapy influences either circulating VWF antigen levels (30) or ADAMTS13 activity.(31) One of the more important conclusions to draw from this pilot study is that early phase CVD patients had lower levels of ADAMTS13 activity **despite** treatment with aspirin therapy at standard therapeutic doses used for secondary prevention of recurrent vascular events. Aspirin has been clearly shown to prolong C-EPI closure times in patients following TIA or stroke,(18, 32) and may well have influenced the analysis of the relationship between ADAMTS13 activity and C-EPI closure times. However, because we have demonstrated that it is highly likely that any apparent relationship between ADAMTS13 activity and C-ADP closure times is mediated via VWF:Ag levels, it is unlikely that any relationship between ADAMTS13 and C-EPI closure times would be of clinical relevance. This study was also performed when the evidence for statin therapy in TIA or ischaemic stroke was emerging,(33) so it will be of interest to see whether these data can be replicated in a larger cohort of patients on secondary preventative therapy with statins.

Conclusions

ADAMTS13 is a dynamic protease whose activity initially decreases and then returns towards baseline levels over time following TIA or stroke. VWF:Ag levels are increased in both the early and late phases after TIA or stroke onset, and can promote enhanced platelet adhesion or aggregation in response to stimulation with collagen-ADP at moderately high shear stress. Further longitudinal studies in larger patient populations on other secondary preventive therapies are warranted to assess the profile of ADAMTS13 activity and VWF sub-fractions following TIA or ischaemic stroke, and its impact on platelet reactivity at different shear rates. This is

especially important in view of the role that ADAMTS13 may have as a potential anti-thrombotic agent in CVD.(34)

Acknowledgements

Dr McCabe and Dr Murphy are **co-first authors** on this manuscript. Dr McCabe's research in this study was funded by the Brain Research Trust, UK. Dr Murphy's research is funded by the Trinity College Dublin Innovation Bursary, the Meath Foundation, Ireland, Joint IICN/Merck Serono Fellowship in Neuroscience, The Vascular Neurology Research Foundation, Ireland, and by an unrestricted education grant from Bayer HealthCare, Ireland, and Roche, UK. Dr Harrison has worked as a Consultant for Sysmex, UK, and received honoraria for presenting PFA-100 data at conferences. Professor Brown's Chair in Stroke Medicine is supported by The Reta Lila Weston Trust for Medical Research, UK. This work was partly supported by researchers at the National Institute for Health Research, University College London Hospitals Biomedical Research Centre. On behalf of all authors, the corresponding author states that there are no conflicts of interest to declare.

References :

1. Bongers TN, de Maat MP, van Goor ML, Bhagwanbali V, van Vliet HH, Gomez Garcia EB, Dippel DW, et al. High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability. *Stroke* 2006;37:2672-2677.
2. Nishio K, Anderson PJ, Zheng XL, Sadler JE. Binding of platelet glycoprotein Iba1 to von Willebrand factor domain A1 stimulates the cleavage of the adjacent domain A2 by ADAMTS13. *Proc Natl Acad Sci U S A* 2004;101:10578-10583.
3. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 2002;100:4033-4039.
4. Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. *Blood* 2008;112:11-18.
5. Zhao BQ, Chauhan AK, Canault M, Patten IS, Yang JJ, Dockal M, Scheiflinger F, et al. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood* 2009;114:3329-3334.
6. Fujioka M, Hayakawa K, Mishima K, Kunizawa A, Irie K, Higuchi S, Nakano T, et al. ADAMTS13 gene deletion aggravates ischemic brain damage: a possible neuroprotective role of ADAMTS13 by ameliorating postischemic hypoperfusion. *Blood* 2010;115:1650-1653.
7. Chion CK, Doggen CJ, Crawley JT, Lane DA, Rosendaal FR. ADAMTS13 and von Willebrand factor and the risk of myocardial infarction in men. *Blood* 2007;109:1998-2000.
8. Crawley JT, Lane DA, Woodward M, Rumley A, Lowe GD. Evidence that high von Willebrand factor and low ADAMTS-13 levels independently increase the risk of a non-fatal heart attack. *J Thromb Haemost* 2008;6:583-588.
9. Schetttert IT, Pereira AC, Lopes NH, Hueb WA, Krieger JE. Association between ADAMTS13 polymorphisms and risk of cardiovascular events in chronic coronary disease. *Thromb Res* 2010;125:61-66.
10. Lambers M, Goldenberg NA, Kenet G, Kirkham FJ, Manner D, Bernard T, Mesters RM, et al. Role of reduced ADAMTS13 in arterial ischemic stroke: a pediatric cohort study. *Ann Neurol* 2013;73:58-64.
11. Andersson HM, Siegerink B, Luken BM, Crawley JT, Algra A, Lane DA, Rosendaal FR. High VWF, low ADAMTS13, and oral contraceptives increase the

risk of ischemic stroke and myocardial infarction in young women. *Blood* 2012;119:1555-1560.

12. Bongers TN, de Bruijne EL, Dippel DW, de Jong AJ, Deckers JW, Poldermans D, de Maat MP, et al. Lower levels of ADAMTS13 are associated with cardiovascular disease in young patients. *Atherosclerosis* 2009;207:250-254.
13. Catto AJ, Carter AM, Barrett JH, Bamford J, Rice PJ, Grant PJ. von Willebrand factor and factor VIII: C in acute cerebrovascular disease. Relationship to stroke subtype and mortality. *Thromb Haemost* 1997;77:1104-1108.
14. Smith FB, Lee AJ, Fowkes FG, Price JF, Rumley A, Lowe GD. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol* 1997;17:3321-3325.
15. De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C. von Willebrand factor: an emerging target in stroke therapy. *Stroke* 2012;43:599-606.
16. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer--PFA-100. *Semin Thromb Hemost* 1995;21 Suppl 2:106-112.
17. Harrison P, Robinson MS, Mackie IJ, Joseph J, McDonald SJ, Liesner R, Savidge GF, et al. Performance of the platelet function analyser PFA-100 in testing abnormalities of primary haemostasis. *Blood Coagul Fibrinolysis* 1999;10:25-31.
18. McCabe DJ, Harrison P, Mackie IJ, Sidhu PS, Lawrie AS, Purdy G, Machin SJ, et al. Assessment of the antiplatelet effects of low to medium dose aspirin in the early and late phases after ischaemic stroke and TIA. *Platelets* 2005;16:269-280.
19. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood* 2001;98:2730-2735.
20. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24:35-41.
21. Yarranton H, Lawrie AS, Mackie IJ, Pinkoski L, Corash L, Machin SJ. Coagulation factor levels in cryosupernatant prepared from plasma treated with amotosalen hydrochloride (S-59) and ultraviolet A light. *Transfusion* 2005;45:1453-1458.
22. Gerritsen HE, Turecek PL, Schwarz HP, Lammle B, Furlan M. Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding

affinity of degraded vWF: a tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP). *Thromb Haemost* 1999;82:1386-1389.

23. Austin SK, Starke RD, Lawrie AS, Cohen H, Machin SJ, Mackie IJ. The VWF/ADAMTS13 axis in the antiphospholipid syndrome: ADAMTS13 antibodies and ADAMTS13 dysfunction. *Br J Haematol* 2008;141:536-544.
24. Starke R, Machin S, Scully M, Purdy G, Mackie I. The clinical utility of ADAMTS13 activity, antigen and autoantibody assays in thrombotic thrombocytopenic purpura. *Br J Haematol* 2007;136:649-655.
25. Reiter RA, Knobl P, Varadi K, Turecek PL. Changes in von Willebrand factor-cleaving protease (ADAMTS13) activity after infusion of desmopressin. *Blood* 2003;101:946-948.
26. Reiter RA, Varadi K, Turecek PL, Jilma B, Knobl P. Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation. *Thromb Haemost* 2005;93:554-558.
27. Muir KW, Tyrrell P, Sattar N, Warburton E. Inflammation and ischaemic stroke. *Curr Opin Neurol* 2007;20:334-342.
28. Dong JF, Moake JL, Bernardo A, Fujikawa K, Ball C, Nolasco L, Lopez JA, et al. ADAMTS-13 metalloprotease interacts with the endothelial cell-derived ultra-large von Willebrand factor. *J Biol Chem* 2003;278:29633-29639.
29. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998;339:1585-1594.
30. Tobin WO, Kinsella JA, Kavanagh GF, O'Donnell JS, McGrath RT, Coughlan T, Collins DR, et al. Longitudinal assessment of von Willebrand factor antigen and von Willebrand factor propeptide in response to alteration of antiplatelet therapy after TIA or ischaemic stroke. *J Neurol* 2014.
31. Matsumoto M, Kawaguchi S, Ishizashi H, Yagi H, Iida J, Sakaki T, Fujimura Y. Platelets treated with ticlopidine are less reactive to unusually large von Willebrand factor multimers than are those treated with aspirin under high shear stress. *Pathophysiol Haemost Thromb* 2005;34:35-40.
32. Tobin WO, Kinsella JA, Coughlan T, Collins DR, O'Neill D, Murphy RP, Egan B, et al. High on-treatment platelet reactivity on commonly prescribed antiplatelet agents following transient ischaemic attack or ischaemic stroke: results from the Trinity Antiplatelet Responsiveness (TRAP) study. *Eur J Neurol* 2013;20:344-352.

33. Amarenco P, Benavente O, Goldstein LB, Callahan A, 3rd, Sillesen H, Hennerici MG, Gilbert S, et al. Results of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial by stroke subtypes. *Stroke* 2009;40:1405-1409.
34. Chauhan AK, Motto DG, Lamb CB, Bergmeier W, Dockal M, Plaimauer B, Scheifflinger F, et al. Systemic antithrombotic effects of ADAMTS13. *J Exp Med* 2006;203:767-776.

Highlights:

- ADAMTS13 activity is significantly reduced in the early phase after TIA/ ischaemic stroke
- ADAMTS13 activity has a distinct inverse relationship with VWF in the early phase
- Positive correlation between ADAMTS13 activity and C-ADP closure times in early phase patients
- ADAMTS13 has future potential as an antithrombotic agent